CLAIM LISTING

1 (Withdrawn) A method for obtaining a cell wall C-polysaccharide antigen containing not more than about 10% protein from the bacterium *Streptococcus pneumoniae* which comprises the steps of:

(a) culturing th bacterium for a time requisite to obtain a sample of desired size and harvesting the bacterial cells therefrom in the form of a wet cell pellet;

(b) suspending the wet cell pellet in an alkaline solution and mixing;

(c) adjusting the pH to an acid pH with a strong acid and centrifuging;

(d) separating the supernatant from step (d) and adjusting its pH to approximate neutrality;

(e) digesting this product with a broad spectrum protease enzyme preparation to destroy residual proteins;

(f) adjusting the pH to the alkaline side with a weakly alkaline aqueous solution

(g) separating out the essentially protein free polysaccharide antigen on a size exclusion column equilibrated with a weakly alkaline solution; and

(h) pooling material eluted in the first peak and adjusting its pH to approximate neutrality.

2 (Withdrawn) The cell wall C-polysaccharide antigen containing not more than about 10% protein obtained by the method of claim 1.

3 (Withdrawn) A method according to claim 1 in which the alkaline solution of step (b) comprises about 20 ml. per gram of said wet cell pellet of 0.1M aqueous sodium hydroxide.

4 (Withdrawn) A method according to claim 1 in which in step (c) the pH is adjusted to about 3.0.

5 (Withdrawn) A method according to claim 1 in which, in step (f) the pH is adjusted to a pH between about 10 and about 11.

6 (Withdrawn) A method according to claim 1 in which, afer step (h), a lyophilization step is performed.

7 (Withdrawn) A method for the purification of raw antibodies to *S. pneumoniae* which comprises the step of:

(a) separating from *S. pneumoniae* bacteria a cell-wall C-polysaccharide antigen containing not more than about 10% protein, and

(b) conjugating said antigen to one end of a two-ended spacer molecule to form a conjugate of said antigen with the spacer molecule;

8 (Withdrawn) Purified antigen-specific antibodies to the cell wall C-polysaccharide of *S. pneumoniae* obtained by the method of claim 7.

9 (Withdrawn) A chromatographic column for affinity purification of raw antibodies to *S. pneumoniae* having coupled thereto by a spacer molecule a purified C-polysaccharide cell wall antigen of *S. pneumoniae* containing not more than about 10% protein.

10-32 Cancelled

33 (Previously Presented) A method of detecting the presence of the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*, in a liquid sample, which method comprises the following steps:

a) culturing *Streptococcus pneumoniae* bacteria, to obtain a desired size of culture and harvesting therefrom cells thereof as a wet cell pellet;

b) separating from the wet cell pellet the cell wall C-polysaccharide antigen containing not more than 10% protein by performing a series of steps which comprises;

(i) suspending the wet cell pellet in an alkaline solution and mixing;

(ii) adjusting the pH to an acid pH with a strong acid;

(iii) separating the mixture from step (ii) into two layers;

(iv) removing the upper layer and adjusting its pH to approximate neutrality;

(v) adding to the product from step (iv) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vi) adjusting the pH of the product from step (v) to an alkaline pH with a weakly alkaline aqueous solution; and (vii) separating out the cell wall C-polysaccharide antigen containing not more than 10% protein;

c) coupling to a chromatographic column through a spacer molecule the cell wall C-polysaccharide antigen containing not more than 10% protein obtained in step (b);

d) passing polyvalent antibodies to *Streptococcus pneumoniae* over the chromatographic affinity column of step (c) to produce purified antigen-specific antibodies; and

e) conducting an immuno-assay upon a liquid sample suspected of containing *Streptococcus pneumoniae* and/or its C-polysaccharide cell wall antigen which immuno-assay comprises the steps of

(i) contacting the liquid sample with conjugates of purified antigen specific antibodies from step (d) hereof and a labelling agent capable of manifesting a color or a detectable signal upon completion of the immunoassay, whereupon C-polysaccharide cell wall antigen of *Streptococcus pneumoniae* in the sample, whether or not in free form, will react with said conjugates to form labelled antibody-antigen conjugates,

(ii) further contacting the liquid and all of the conjugates it contains with a solid surface upon which a mass of unlabelled antigen-specific antibodies from step (d) hereof have been immobilized, whereupon any labelled antibody-antigen conjugates present will react with the immobilized antibodies on the surface to form labelled antibody-antigen-immobilized antibody sandwiches, and

(iii) detecting any label thereby accumulated on the solid surface by a detection means appropriate to the nature of the label so as to confirm the presence of the *Streptococcus pneumoniae* C-polysaccharide cell wall antigen in the sample.

34 (Previously Presented) The method of claim 33 in which the spacer molecule of step (c) is a protein molecule.

35 (Previously Presented) The method of claim 33 wherein the sample of step (e) is a natural liquid of mammalian origin.

36 (Previously Presented) The method of claim 35 wherein the liquid sample of step (e) is human urine.

37 (Previously Presented) The method of claim 36 in which the liquid sample is taken from a patient exhibiting clinical signs of pneumonia.

38 (Previously Presented) The method of claim 36 in which the liquid sample is taken from a patient exhibiting clinical signs of otitis media.

39 (Previously Presented) The method of claim 35 wherein the liquid sample of step (e) is human spinal fluid.

40 (Previously Presented) The method of claim 39 wherein the sample is obtained from a patient suspected of having meningitis.

41 Cancelled

42 (Previously Presented) The method of claim 33 in which step (e) is an immunochromatographic ("ICT") process.

43 (Previously Presented) The method of claim 42 in which step (e) is conducted by

a) contacting a liquid sample suspected f containing *Streptococcus pneumoniae* and/or its free cell wall C-polysaccharide antigen, with the sample-receiving end of a strip of bibulous material, which strip is contained within an ICT device comprising a housing and itself comprises

(i) a first zone in which has been movably embedded a conjugate of a labelling agent with purified antigen-specific antibodies obtained in step (d) of claim 33, said labelling agent being selected from among those known to manifest a visible color change upon the formation of a labelled antibody-antigen-fixed antibody reaction product and

(ii) a second zone having fixedly bound thereto a stripe of unconjugated purified antigen-specific antibodies from step (d) of claim 33, which zone is equipped with a window in the housing for viewing the appearance of a color characteristic of the massing of the labelling agent upon the formation of the labelled antibody-antigen-fixed antibody reaction product;

b) allowing said liquid sample to flow laterally along said test strip to said first zone where it picks up the movably embedded conjugate of labelling agent and antigen-specific antibodies obtained in step(d) of Claim 33

c) allowing said liquid sample and said conjugate of antigen-specific antibodies to flow laterally together along said test strip to said second zone while concomitantly reacting to form labelled antibody-antigen conjugates with C-polysaccharide cell wall antigen of *Streptococcus pneumoniae*, free or combined, present in the sample and

d) within not more than 20 minutes after first contacting the liquid sample with the test strip, observing, through said window in the housing whether a line of color has formed, indicative of the massing of said label along the stripe of unconjugated purified antibodies, as labelled antibody-antigen-fixed antibody reaction products are formed.

44 (Previously Presented) The method of claim 43 wherein the sample is a natural liquid of mammalian origin.

45 (Previously Presented) The method of claim 44 wherein the sample is human urine.

46 (Previously Presented) The method of claim 45 wherein the sample is taken from a patient exhibiting overt clinical signs of pneumonia or another respiratory tract illness known to be often caused by *Streptococcus pneumoniae*.

47 (Previously Presented) The method of claim 44 wherein the liquid sample is human spinal fluid.

48 (Previously Presented) The method of claim 45 wherein the liquid sample is taken from a patient exhibiting clinical signs of otitis media.

49 (Previously Presented) The method of claim 45 wherein the liquid sample is taken from a patient suspected of having meningitis.

50 (Currently amended) An immunochromatographic ("ICT") device for the detection of the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae* in a liquid sample, which device comprises a housing equipped with a window and containing a strip of bibulous material, ~~which strip of bibulous material has~~

at least having at least a first zone and a second zone, said strip being so positioned within said housing that its second zone appears directly beneath said window, said strip being further characterized in that

a) ~~a first zone in which has been~~ said first zone has movably embedded therein a conjugate of a labeling agent and purified antibodies specific to the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*, and

b) said second zone is located ~~a second zone~~, downstream of said first zone ~~and equipped with a window in the housing~~, to which second zone is immovably bound a stripe of unlabelled purified antibodies specific to the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*,

wherein all of said antibodies specific to the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae* in both zones have been obtained by passing polyvalent antibodies to *Streptococcus pneumoniae* over a chromatographic affinity column to which is coupled ~~a spacer molecule conjugated to~~ a purified cell wall C-polysaccharide antigen of *Streptococcus pneumoniae* obtained from a culture of *Streptococcus pneumoniae* bacteria according to a method comprising the steps of ~~the following method:~~

(i) harvesting cells from the said culture in the form of a wet cell pellet;

(ii) suspending the wet cell pellet in an alkaline solution and mixing;

(iii) adjusting the pH of the resultant mixture to an acid pH with a strong acid;

(iv) separating the acidified product from step (iii) into two layers;

(v) removing the upper layer and adjusting its pH to approximate neutrality;

(vi) adding to the product from step (v) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vii) adjusting the pH of the product from step (vi) to an alkaline pH with a weakly alkaline aqueous solution; and

(viii) separating out the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae* having no more than 10% protein.

51 (Previously Presented) The ICT device of claim 50 wherein the labelling agent is finely divided metallic gold.

52-54 Cancelled